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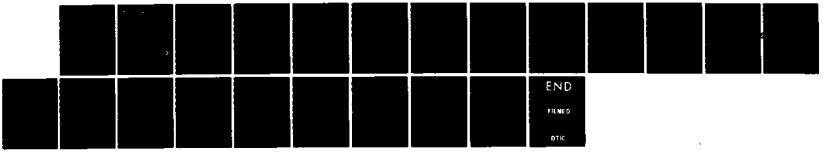

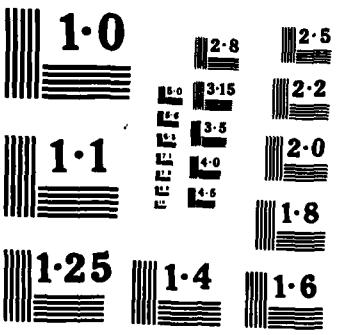
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ONRL Report C-1-85

Third European Congress on Biotechnology

Claire E. Zomzely-Neurath

21 February 1985

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The Third European Congress on Biotechnology was held in Munich, West Germany, from 10 through 14 September 1984. This report examines trends in biotechnology suggested by the congress as well as the congress papers on thermophilic microorganisms, biosurfactants, immobilized cells and enzymes, applied genetics, food and feed bioprocesses, and fine chemicals and pharmaceuticals--bioprocesses and downstream processing. Originator Supplied Keywords include:

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CONTENTS

THIRD EUROPEAN CONGRESS ON BIOTECHNOLOGY

1 INTRODUCTION

Biotechnology has become one of the most rapidly expanding fields of science and technology. Modern scientifically based interdisciplinary biotechnology has developed since the early forties. As defined by the European Federation of Biotechnology, "Biotechnology is the integrated use of biochemistry, microbiology and engineering sciences in order to achieve the technological application of the capabilities of microorganisms, culture tissue cells, and parts thereof."

One of the major meetings in the field was the Third European Congress on Biotechnology, held in Munich, West Germany, from 10 through 14 September 1984. The congress was organized by DECHEMA (Deutsche Gesellshaft für Chemisches Apparatewisen, or Germany Society for Chemical Instrumentation, Frankfurt am Main, West Germany). The Technical University of Munich, West Germany, was the site of the meetings.

Background

The scientific program consisted of six plenary lectures by invitation, eight review/preview lectures also by invitation, oral presentations and poster sessions on application and fundamental topics, special sessions, and workshops. (A list of all topics appears in the appendix, page 18.) Although 600 papers were submitted, the program was limited to 350 general papers, 94 presented in oral sessions and 256 in poster sessions. The number of general papers had to be limited because of insufficient space in the conference building of the university. Copies of the abstract books, available at the meeting, as well as the proceedings, can be ordered from the publisher, Verlag Chemie GmbH, Pappelallee 3, D-6940, Weinheim, West Germany. (Plenary lectures and review/preview papers were not available at the time of the meeting.)

The total attendance (preregistered participants) was 1523. However, apparently a few hundred attendees registered during the week of the meeting, so that the final tally was about 2000. Eight-two percent of the attendees were from West European countries, with the largest representation from West Germany followed by Holland, the UK, and Switzerland. The remainder (12.1 percent) came from widely diverse areas such as the US and Canada; Japan; Israel; Middle Eastern countries (e.g., Iraq, Egypt); and Australia. Of this latter group, the largest number was from Japan. Academic institutions accounted for 42.3 percent of the total attendance, and 57.7 percent from industry and industry-related institutions.

Trends in Biotechnology

Since the number of plenary lectures, special sessions, and workshops at the congress was extensive, an overview of the salient issues raised by the various speakers will be presented at this time. In the first place, it should be emphasized that biotechnology has evolved, of necessity, as a cooperative effort between the biosciences and the engineering disciplines to develop technological applications. The public perception of biotechnology is highly influenced these days by widespread early publicity of successes in research based on new and elegant genetic methods. This has led to overoptimism that biotechnology is a panacea for many of the world's problems and to an incorrect identification of biotechnology solely with genetic engineering using recombinant DNA techniques. Although, in a strict sense, biotechnology is mainly involved with industrial and environmental processes, the application in plant and animal agriculture as well as in human health of biotechnological products is of great importance, and the trend toward an extension to the latter areas will assume increasing emphasis. Thus, the point was made that there should be additional implementation of biotechnology courses at universities which would integrate biosciences and engineering disciplines.

Many European universities have already started such programs. The era of new biotechnologies is characterized

by three innovations--genetic engineering, hybridoma technology, and bioprocess engineering--which, in the opinion of the various speakers, will determine the biotechnology of the next decade. The consensus was that the consequences, especially of genetic engineering, will reach beyond biotechnology in industry and in environmental processes, to broader areas of application such as agriculture and medicine. Extensive work is already being done in the last two areas, although still mostly at the level of basic research. At the present time, however, most of the money for biotechnology is spent by producers of beer and wine (fermentation technology). These classical products still represent more than 75 percent of the turnover of all biotechnological products; while in terms of the total amount of sales, environmental biotechnology is by far the largest application of biotechnology processing.

Several speakers emphasized an important point: although the great potential of recombinant DNA technology has put biotechnology as a whole into the limelight, genetic engineering is only a *part* of biotechnology. The attraction of molecular biology for students and researchers was considered to be potentially detrimental to the interests in other disciplines such as microbial physiology and the fields of biochemical isolation and purification, which are regarded as less glamorous. This may pose a threat to a harmonious further development of biotechnology as an interdisciplinary science.

Data were presented showing that Europe has a competitive edge--in terms of high export positions--in the industrial sectors of fermentation products, pharmaceuticals, alcoholic beverages, and food/feed. It seems that Denmark, Belgium, West Germany, France, The Netherlands, Switzerland, and the UK are the exporting countries with a broad representation in the high-tech biotechnologies (fermentation, pharmaceuticals food/feed industry, fine chemical and biotechnological equipment). In the low-tech field of the more traditional biotechnologies (alcoholic beverages, food), the following countries have high export positions: Denmark, France, Italy, The Netherlands, Switzerland, and the UK. The total picture indicates that in addition to West Germany, France, Italy, and the UK, smaller countries which have a strong overall position are Denmark, The Netherlands, Switzerland, and Belgium.

Venture capital investments (high risk) in start-up biotechnology companies have become a characteristic trait of the industrial scene in the US. In Europe, this form of entrepreneurship lags far behind. About 80 percent of the venture capital companies in biotechnology are of US origin; the remainder are to be found mainly in the UK, with some in France, Belgium, Sweden, and The Netherlands. West Germany, Italy, Denmark, and Switzerland do not have such investments. This has been attributed to various causes, such as the national banking system, the absence of entrepreneurship, insufficient expertise, and national tax systems which do not favor high-risk undertakings. The US has, at present, a competitive advantage in the commercialization of biotechnology due to a well-developed life science base, the availability of financing for high-risk ventures, and an entrepreneurial spirit. Japan is considered likely to be the leading competitor to the US for two reasons: (1) a broad range of industrial sectors have extensive experience in bioprocess technology, and (2) the Japanese government has targeted biotechnology as a key technology for the future and thus is funding its commercial development and coordinating interactions among representatives from industry, universities, and government. European countries (West Germany, France, the UK, and Switzerland) are not moving as rapidly as Japan and the US to commercialization of biotechnology and are not expected to be as strong competitors in biotechnology. The European countries generally do not promote risk taking, either in industry or in government policies, and they have fewer companies commercializing biotechnology.

However, in markets for specific products--including some pharmaceuticals, specialty chemicals, and animal agriculture products--some European companies are expected to be strong international competitors.

The main chances for European biotechnology were considered to be in pharmaceuticals and fine chemicals. In the pharmaceutical industry, new technologies such as cell fusion to make hybridomas and genetic engineering are expected to have their first impact in: (1) improved production of existing products, e.g., improved antibiotic yields; (2) therapeutics; (3) prophylatics; and (3) diagnostics. The fine-chemical industry should further exploit the specificity of biosynthesis for the production for a wide variety of complex speciality chemicals, such as enzymes, flavors, stabilizers, insecticides, surfactants, materials for tertiary oil recovery, and steroids. The search for products made in plant cell cultures is an active area of research, mainly in Europe and Japan, with the expectation that some products may become competitive as pharmaceuticals or fine chemicals. Biotechnology should also make substantial contributions to the production of feed stuffs and food as well as to environmental processes. Fish production in Europe will be centered around single cell protein, either on a large industrial scale or by smaller local plants converting agricultural wastes of cellulosic substances. The strategic incentive is to reduce the dependence on imports of proteineous feed (e.g., soya). Environmental processes should lead to increased availability of resources from recycling. Anaerobic processes will be supplied more widely, using new generations of reactor systems and thus partially meeting local needs for food stuffs and foods at the same time. It will also be necessary to develop detoxification contaminants that now prevent full recycling.

In plant agriculture, *in vitro* plant cell cultures for selection and regeneration of improved and homogeneous plant varieties is being used increasingly in plant breeding and propogation. The method is now routinely applied in horticulture and for certain agricultural crops, including oil palms. Unilever's UK research labs, for example, have developed this methodology for improving oil palm plantations in Southeast Asia. The application of recombinant DNA technology offers the possibility of combining genetic material from plants that normally do not inbreed, and this could provide simultaneous introduction of many specific traits into a single breeding line. Other possibilities utilizing the new technologies are: (1) introduction and expression in plants of genes conferring resistance to stress and disease; (2) changing the composition of storage proteins in cereals and legumes; (3) new strains and accelerated breeding programs for floriculture and forestry; (4) improved plant fibers and enzymes for industry; (5) increased biomass production for "energy farming"; (6) insertion of genes for nitrogen fixation directly into the plant genome; (7) microbially produced insecticides; and (8) genetically altered bacteria, devoid of the capacity to make compounds that initiate ice crystals for use in replacing related bacteria that are normally present in order to give protection against frost damage. Some of these possibilities are already in the research stage.

Biotechnology can also be used to improve animal feed, nutrition, and health, with the aim of improving production of food from animals. The various approaches are: (1) health improvement by the use of monoclonal-based diagnostics, improved or new vaccine--e.g., foot and mouth disease, rabies, fowl plague; (2) passive immunization with monoclonal antibodies; and (3) increased productivity by promoting animal growth--e.g., animal growth hormones.

The application of biotechnology from the level of basic research to practical, commercial application is a relatively slow process, but taken about the same time as in, for example, the

field of synthetic drugs and antibiotics. European countries have, to a large extent, made major commitments toward implementing existing programs to increase their use of biotechnology, particularly with respect to the new technologies.

2 CONFERENCE PAPERS

This chapter discusses selected topics from the 350 oral and poster sessions.

Thermophilic Microorganisms

A. Fontana (Institute of Organic Chemistry, Biopolymer Research Center, University of Padua, Italy) presented a general view of thermophilic enzymes as well as some of the research from his laboratory. The interest of biotechnologists in thermophilic microorganisms is their potential use in biotechnology. Biotechnological applications of enzymes are often hampered by the intrinsic liability of enzymes to heat, organic solvents, proteolysis, etc. A significant improvement to existing enzyme technology would be the use of enzymes isolated from thermophilic microorganisms. These enzymes are much more resistant to heat and other protein-denaturing agents than their counterparts from mesophilic sources. During the past 10 years, a large number of enzymes have been purified from thermophilic microorganisms, and their functions and molecular properties have been studied. The initial focus of researchers was on increasing the thermostability of these enzymes and finding the molecular mechanisms responsible for this unusual stability. More recently, however, the attention of many researchers has been directed toward the potential use of the enzymes in biotechnology.

A large variety of enzymes has been isolated from *B. stearothermophilus*, which is a moderate thermophile growing optimally at 60°C to 65°C. More thermostable enzymes have been obtained from thermophilic microrganisms such as *Thermus thermophilus*, *Thermus aquaticus*, and *B. caldotenox*, which grow optimally at 70°C to 75°C. Thermolysin, isolated from *B. thermoproteolyticus* is presently the most well-characterized thermostable neutral protease. More recently, an extracellular metallo-protease, called caldolysin, has been isolated from *Thermus T-351*. Enolase (a glycolytic enzyme) obtained from *Thermus aquaticus* and *Thermus X-1* has been shown to be highly thermostabile and to possess unusual quaternary structure. This enzyme consists of eight apparently identical subunits of 44,000 to 48,000 molecular weight, whereas the enzymes from yeast, rabbit muscle, rat muscle, liver, brain, and heart are dimers. Enolase from *B. stearothermophilus* is octomeric.

In a poster session, M. de Rosa and A. Gambacorta (Institute of Chemistry, Consiglio Nazionale delle Richerche [CNR], Arco Felici, Naples, Italy) in collaboration with V. Buonocare (Institute of Organic and Biological Chemistry, Faculty of Sciences, University of Naples) presented studies on an extremely thermophilic archaebacterium, *Sulfolobus solfataricus* (previously named *Calderiella acidophila)*, which grows optimally at 87°C, pH 3.5. The enzymes that were studied for biotechnological applications were: beta-galactosidase, DNA polymerase, malic enzyme, and some enzymes related to glucose metabolism (such as glucose dehydrogenase, gluconate dehydrogenase, aldolase). All enzymes were purified and characterized. These investigations also presented some data on immobilization of free enzymes and whole cells in different polymeric matrices. This poster session attracted a lot of attention from attendees at the conference because of the extreme stability of this microorganism as well as the large amount of data presented on the characterization of the enzymes isolated from *Sulfolobus solfataricus*.

E. Parkkinin and M. Korhola (Research Laboratories of the Finnish State Alcohol Co., Alko Ltd., Helsinki, Finland) studied the conversion of starch to ethanol by *Clostridium thermohydrosulfuricum* using soluble and insoluble starch. This microorganism is one of the few thermophilic anaerobic

bacteria known to grow on starch and to ferment it. This anaerobe ferments a great variety of carbohydrates, including starch sucrose, cellobiose, glucose, and xylose to ethanol, carbon dioxides, lactate, acetate, and hydrogen gas. The amount of growth and specific fermentation product yields of *Clostridium thermohydrosulfuricum* in complex media varies with the kind of sugar fermented. Product yields vary also with the concentration of substrate fermented. The purpose of this study was to investigate optimal conditions for starch fermentation that would favor ethanol production over other products.

G. Kvesitadze, L. Kvachadaze, T. Aleksidze, and T. Buachidze (Institute of Plant Biochemistry, Georgian Academy of Sciences, Tbilisi, USSR) presented data on thermophilic micromicetes which are producers of cellulases. They found 12 strains which were considered to be thermotolerant with optimal growing temperatures of 40°C to 50°C. The original variation of thermotolerant cultures was studied and allowed for the selection of the most active cellulase producers. The extracellular components of the thermotolerant strains was also established. Endoglucanese, another cellulose component, similar to cellobiase (i.e., hydrolyzing low molecular fragments to glucose) has been found in nearly all cases. Endoglucanese of *Sporotrachums pulverselentem* and cellobiase of *Aspergillus terreus* were obtained in a highly purified state, and some of the physicochemicals properties of these enzymes were presented. The preparations of cellulase, isolated from the thermotolerent cultures, were characterized by higher thermostabile properties in comparison with cellulase from mesophilic fungi. These investigators found that endoglucanase from thermotolerant micromicetes always exceeded the thermostability of the mesophilic analogues. The preparations, isolated from thermotolerant micromicetes, were able to hydrolyze substrates for a long time at 60°C to 65°C.

A report on the physiology and genetics of a thermophilic carbon monoxide-utilizing bacterium was presented by C.M. Lyons, J. Colby, and E. Williams (Department of Microbiology, Newcastle upon Tyne University Medical Schools, UK, and the Biology Department, Sunderland, Tyne and Wear, UK). The research efforts of these investigators have been directed at isolating novel types of carboxybacteria, with particular emphasis on thermophilic species. One of their isolates, *Pseudomonas thermocarboxydovorana* (NCIB 11893; type strain C2) is a gram-negative, aerobic, thermophilic carboxybacterium from sewage. The organism grows well in mineral medium under an atmosphere of 25 to 80 percent (V/V) carbon monoxide in air. The organism is a facultative autotroph able to grow in a variety of organic acids and amino acids, but unable to grow in sugars. If differs from the mesophilic carboxydotropic bacteria in its optimum growth temperature of 75°C to 85°C, the high G^+C ratio of its DNA (72 mol. percent) and its inability to grow as a hydrogen bacterium, to oxidize hydrogens, or to synthesize hydrogenase. Lyons et al. are interested in using this organism to obtain a cheap enzyme-based sensor able to detect toxic levels of carbon monoxide, or an enzyme-based filter to protect the quality of breathable air. Carbon monoxide is a colorless, odorless, and very toxic gas because of its ability to bind metal proteins. As a major component of fossil fuels, it is a potential hazard both in industry and in the home. Sensors and filters could be based on a carbon monoxide-oxidoreductase from carboxydobacteria. The thermophilic strain has the advantage that this enzyme is heat-stabile with an optimum temperature of 80°C. These investigators are presently attempting to clone the enzyme.

There appear to be a number of advantages for the practical applications of thermophilic enzymes in biotechnology. They are active at high temperatures, and therefore higher reaction rates can be obtained than under meosphilic conditions. Infections are less likely to occur at the operation

emperature; hence, a longer useful ifetime can be obtained. Due to greater olubility of reactants and reduced vis-osity at higher temperatures, higher roductivities at lower cost should be chieved. High thermostability of en-ymes is often coupled with increased esistance to chemical denaturation. uch enzymes should therefore be appli-able in nonaqueous environments. The conomy of thermophilic bioprocesses reatly depends on the advantages one an gain from high temperatures and on he values of the products. Therefore, ne has to assess to what extent the bove-mentioned advantages are realis-ic. This question was addressed in a ecture by B. Sonnleitner, B. Grueniner, R. LaForce, U. Baier, and A. ischtner (Swiss Federal Institute of echnology, Zurich, Switzerland). These nvestigators have been working for everal years with extremely thermo-hilic bacteria or products from them, o the scientists are well qualified to ssess the utility of thermophilic orga-isms for biotechnological processes. hese researchers presented studies on wo different types of thermophilic bio-atalysts: alpha-amylases from extreme hermophilic *Bacilli* as an example of an xoenzyme used in large quantities, and n *Thermoanaerobium brockii* with its ersatile alcohol-aldehyde/ketone oxido-eductases as an example of highly pecialized biocatalysts used in small uantities. The results of these studies howed that the advantages of the use of hermophilic enzymes as biocatalysts are ealistic to a large extent, but the ollowing considerations are important n evaluating the practical application f these biocatalyst:

1. High reaction rates. One has o take care of the mode of process con-rol and of metabolic regulation which oth may not allow the operation at ighest rates.

2. High solubility of reactants. onnleiter et al. consider that so far ne cannot exploit this theoretical dvantage because all extreme thermo-hiles investigated were found to be subject to more or less pronounced inhi-bition by organic matter at concentra-tions which do not inhibit most meso-philic organisms.

3. Infection risk. The infection risk certainly does decrease with increasing cultivation temperature, but it does not disappear.

4. Useful lifetime. The expecta-tion of longer useful lifetime was found so far to be true, especially if appli-cation temperatures did not exceed the optimal temperature.

However, the researchers stressed the importance of a constant availabil-ity of energy to intact cells. Even short periods of energy depletion (some-times only a few minutes) can reduce viability or reactivity seriously, pre-sumably due to the relatively high main-tenance requirements of thermophiles. Sonnleiter et al. also suggested that thermophilic organisms offer a very good source of new bioproducts--some with so-far unknown properties, and some which catalyze certain reactions more effi-ciently and/or more specifically than so-far known mesophilic bacteria.

Biosurfactants

Numerous microbial cultures are capable of synthesizing a variety of biosurfactants. The most commonly iso-lated and characterized biosurfactants are glycolipids, lipopeptides, or lipo-polysaccharides. These biosurfactants are produced from carbohydrates as well as from scarcely water-soluble lypophil-ic carbon sources. In the latter case, the ionic or nonionic surfactants pro-duced by growing cultures of microorga-nisms are involved in the mechanism for the initial interaction of the lipophil-ic substrate with the microbial cell. F. Wagner, J.S. Kim, S. Land, Z.Y. Li, G. Marwede, U. Matulovic, E. Ristau, and C. Syldatk (Institute of Biochemistry and Biotechnology, Technical Universi-ty, Braunschweig, West Germany) studied *Rhodococcus erythropolis* and *Pseudomonas spec.* They described: (1) the influences of culture conditions on the qualitative and quantitative composition of the

microbial lipid mixtures; (2) the production of rhamnolipids with immobilized cells; and (3) the surface and interfacial active properties of isolated trehalose and rhamnose lipids. These investigations found that the use of a high carbon/nitrogen ratio--i.e., nitrogen deficiency--led to a significant increase of the glycolipid yield compared to cultivations under nitrogen saturation. With *Rhodococcus erythropolis* the production could be further improved by a temperature shift from 30°C to 21°C at the beginning of the nitrogen limitation.

The highest rhamnolipid production of *Pseudomanas spec.* DSM 2874 cells was obtained by immobilization with calcium malginate (Manugel DJX, 3 percent, produced by Kelco/AIL, Hamburg, West Germany). These studies as well as other presented showed that: (1) the production of glycolipids is under nitrogen regulation. The conversion rates (gram of product divided by gram of carbon substrate) were markedly increased under "resting" cell conditions; (2) by variation of the reaction conditions, the product composition and, with that the physical properties, could be controlled; (3) all glycolipids were shown to have good surfactant properties in the presence of high salt concentrations. They reduced the interfacial tension against n-hexadecanes at <1 mM/m at critical micelle concentrations of 5 to 200 mg/mL and at T=20°C to 90°C.

B. Frautz, S. Lang, and F. Wagner (Institute of Biochemistry and Biotechnology, Technical University of Braunshweig, West Germany) reported on biosurfactant production by *Ustilago maydis*. This microorganism produces a mixture of extracellular cellobiose lipids called ustilagic acids when grown on different carbohydrates, especially on glucose. These investigators examined the production of glycolipids by *Ustilago maydis* ATCC 14826 on lipophilic substrates with growing and resting cells. They also studied the surface and interfacial properties of the glycolipids. Their results showed that:

1. Lipophilic substances like vegetable oils are suitable substrates for the growth and the glycolipid formation of *Ustilago maydis*.
2. The production phase can be separated from the growth phase. A glycolipid formation can be observed in a medium without nitrogen source.
3. The mixture of glycolipids shows good surfactant properties with critical micelle concentrations in the range of 20 mg/L.

A report on process development for the production of biosurfactants was presented by L. Guerra-Santos, O. Käppeli, and A. Fiechtner (Institute of Biotechnology, Swiss Federal Institute of Technology, Zurich, Switzerland). Rsan-ver, a strain of *Pseudomonas aeruginosa* isolated at the institute, was used for the development of a process for biosurfactant production. The goal of these investigators was to develop a continuous production process based on glucose as the carbon source rather than the usual hydrocarbon substrates, which require more sophisticated equipment and more power input in order to achieve an adequate dispersion of the hydrocarbons. In addition, the availability of hydrocarbons is limited if applications of biosurfactants other than in enhanced oil recovery are envisaged.

The continuous production process has several advantages over production of biosurfactants in batch cultivations: (1) long-term incubation periods of several days are avoided, yielding a much improved productivity per unit reactor volume; (2) there is a constant mass flow which can be adapted to the capacity of the downstream processing; and (3) the exact control of the culture conditions which is essential for high biosurfactant formation by the cells is accomplished more easily in a continuous culture.

Although investigations on biosurfactant production by *Pseudomonas aeruginosa* and on identification of the active compounds as rhamnolipids are known, little information was available

he nutritional requirements of the ıism for optimal glycolipid forma- Guerra-Santos et al., therefore, devised a chemically defined medium high potencies for biosurfactant ıction in continuous culture. They able to obtain a 7- to 10-fold :r concentration of rhamnolipids :essed as rhamnose content per :) and expect that this yield can be :ased by further process development :rain improvement.

›ilized Cells and Enzymes

Immobilized cells have become one he focal points of modern biotech- gy. Their advantages over free cells now well established, and they have introduced in several industrial esses. Immobilization can eliminate of the disadvantages inherent in cell cultures, such as low produc- ty, instability of cell structure, metabolic activities as well as low th rate.

A. Leuchtenberg, C. Wardsack, H. ersdörfer, and H. Ruttlof (Bergholz- rücke, East Germany) presented a y on the production of pectinases mmobilized mycelium of *Aspergillus r.* The basis for the research is increased processing of fruits and tables requires new technological edures, including the application of inolytic enzymes resulting in higher ds and an improved quality of the ucts. For the production of fruit vegetable macerates, pectinolytic mes are needed which contain poly- cturonase (PG) as the main compo- . These enzymes are produced primar- by molds, above all by *Aspergillus r.* Preliminary experiments had n that the strain *Aspergillus niger* 14 produces difficult amounts of gulacturonase depending on mycelium hology. The aim of this study re- ed at the congress was to compare metabolism of different morphologi- growth variants and to obtain rmation for the development of a tical and economical procedure for galacturonase production. Their lts showed that the production of compact mycelium with a higher enzyme synthesis and activity was possible by immobilization of mycelium on a texile matrix. The relation between matrix surface and medium volume greatly influenced PG synthesis. Furthermore, aggregation of mycelium, which is necessary for a high PG production, is possible utilizing growth of mycelium on a matrix. Preliminary data showed that a scaling up of the PG production by immobilized mycelium of *Aspergillus niger* $R_{1/214}$ was possible.

Immobilization of plant cells and its application to pigment production was studied by A. Tanska, K. Sonomoto, N. Msui, H. Nakajiima, S. Fukai, F. Sato, and Y. Yamada (Department of Industrial Chemistry, Faculty of Agriculture, Kyoto University, Japan). Recently, immobilization of plant cells has attracted much interest because plant cells can synthesize *de novo* various useful compounds which are difficult or impossible to be produced by microbial cells. Also, plant cells can catalyze different types of bioconversions specific to themselves. Immobilization of plant cells was used to stabilize and enhance the productivity of the cells. These researchers used cultured cells of *Lavandula vera* strain L_{10} 4-2154, which produces blue pigments for their experiments. Several immobilization methods were tried. Agar-entrapped cells synthesized the highest amount of the pigments but the researchers selected calcium alginate as the gel material based on cell growth, mechanical strength of gels, and low leakage of the cells from the gels. When *Lavandula vera* cells were used repeatedly for the production of pigments, the immobilized cells could be used for at least eight batches over 7 months in contrast to less than 1 month when free cells are used. Therefore, it was shown that immobilization of cells is very effective in stabilizing plant cells for the synthesis of complex metabolites such as pigments via complicated pathways.

B. Kopp, A.H. El-Sayed, W. Mahmoud, and H.J. Rehm (Institute for Microbiology, University of Munster, West

rmany) presented studies on the pro-
ıction of ergot alkaloids, penicillins,
ıd chlortetracycline by immobilized
·ganisms. Three mycelia-forming micro-
·ganisms (*Claviceps purpurea, Penicil-
ium chrysogenum*, and *Streptomyces aure-
faciens*) were immobilized in different
ılcium alginate concentrations, and
ɔmpared to free cells to investigate
ıeir production capacity for ergot
lkaloids, penicillins, and chlortetra-
ycline. By varying and optimizing the
ılture conditions, the amounts of these
ɛcondary metabolites, of pharmaceutical
ıterest, could be increased several
imes. Under special culture conditions,
mmobilized mycelia-forming microorga-
isms only grow below the surface of the
el beads (matrix), but not in the fer-
entation broth, so that excreted sec-
ndary metabolites can be extracted from
he medium more easily than from fermen-
ations with free cells. Thus, process-
s with immobilized microorganisms are
f value.

Immobilization of microbial cells
y adhesion to a support was investiga-
ed by P.G. Rouxhet, N. Mozese, J.L. Van
aecht, L. Reuliaux, and M.H. Palm-
ennen (Group of Mineral Physical Chem-
stry and Catalysis, Catholic University
f Louvain, Belgium). Immobilization of
hole cells by adhesion (also called
dsorption) on a carrier was studied,
ith emphasis on a direct study of the
dhesion process. The particle-particle
nteractions or particle-macroscopic
ody interactions comprise two additive
erms: one due to van der Waals forces,
nd one due to electrostatic forces.
tarvation of yeast cells (*Saccharmyces
erevisiae*) in pure water provokes their
dhesion to glass or polycarbonate sup-
orts. The efficiency of the treatment
as shown to be related to both a re-
ease of substances, decreasing the
ell-cell and cell-support electrostatic
epulsions, and a modification of the
ell wall itself. Adhesion of yeast to
lass was also achieved by acting
irectly on electrostatic interactions:
dsorbing metallic ions on the cells or
n the support, or coating the support
ith a layer of positively charged
colloidal particles which act as a bind-
ing agent between the cells and the sup-
port. Several microorganisms (*Xant-
hommas compestris, Arthobacter simplex,
Acetobacter aceti, E. coli* [A223], and
Moniliella tomentosa) were studied in
addition to yeast cells. Rouxhet et al.
found that adhesion of the microfungus
Moniliella to glass and polystyrene
plates as supports was increased as a
function of pH (i.e., as the pH de-
creased from 5 to 3.0). This was attri-
buted to a decrease of the surface nega-
tive charges of both the cells and the
support, leading to a reduction of elec-
trostatic repulsion. Trials to obtain
adhesion of the other cited microorga-
nisms in various supports by simple pH
adjustment were not successful. The
authors suggested that the difference
between *Moniliella tomentosa* and the
other microorganisms studied lies pre-
sumably in the balance of the nonelec-
trostatic intermolecular forces at the
cell-water-support interface. The adhe-
sion of the various microorganisms to
glass or polycarbonate plates, glass
beads, or fibers could be enhanced by
treatment of these cells with Al^{3+}, Fe^{3+}
or binding with colloidial particles (Al
$(OH)_3$ Fe_2O_3).

S. Birnbaum, L. Bülow, K. Hardy, S.
Stahl, J. Davies, and K. Mosback (Pure
and Applied Biochemistry, Chemical Cen-
ter, Lund, Sweden, and Biogen S.A.,
Geneva, Switzerland) presented a report
on protein production by immobilized
genetically engineered bacteria. These
researchers showed that recombinant
Bacillus subtilis cells were able to
produce proinsulin continuously in the
immobilized state. They also investi-
gated immobilized *E. coli* cells pro-
ducing proinsulin. They found that cer-
tain antibiotics which inhibit cell
growth were effective in reducing cell
leakages from the immobilized cell pre-
paration and that these entrapped *E.
coli* cells still produced proinsulin.
Thus, they have applied immobilized cell
technology in conjunction with the
recombinant DNA technique for the pro-
duction of proteins to achieve greater
efficiency and product yield.

Direct enzyme stabilization within an ultrafiltration membrane vector was studied by L. Gianfreda, R. Langella, and G. Greco Jr. (Faculty of Pharmacy and Department of Chemical Engineering, University of Naples, Italy). Since industrial applications of enzymatic proteins as biocatalysts is often limited by their intrinsic lability, stabilization techniques of fairly general applicability must be developed. These researchers investigated the enhancement in enzyme stability achieved by making joint use of a completely polarized ultrafiltration membrane reactor and of linear-chain water soluble synthetic polymers. An ultrafiltration cell can be employed as an immobilized enzyme reactor since enzyme confinement is a direct consequence of the rejective properties of the membrane. In a steady-state, unstirred plane membrane system, virtually all of the protein is confined immediately upstream from the membrane, within a volume whose dimension depends on permeate flow rate and on enzyme diffusivity. If the enzyme is injected into the reactor together with a linear-chain soluble macromolecule, they both accumulate at the membrane surfaces, where extremely high macromolecular concentration levels are obtained. Thus, a polymeric network is produced, wherein the protein mobility is virtually reduced to nil, producing considerable enhancement in enzyme thermal stability. Granfreda et al. presented data on the kinetics and the thermodynamics of the deactivation process, applying the stabilizing technique described above.

B. Bisping and H.J. Rehm (Institute for Microbiology, University of Munster, West Germany) presented a paper on the production of glycerol by immobilized yeast cells. They found that yeast cells immobilized in polyacrylamide-hydrazide had the following advantages in contrast to cells immobilized in calcium alginate gels (the usual procedure): (1) the gel particles remained stable in the presence of high ionic concentrations; (2) the titre of cells growing out of the gel was reduced; (3) the time of fermentation was reduced; and (4) the cells immobilized in polyacrylamide-hydrazide could be reused several times, a fact which is a precondition for the buildup of a continuous system.

H. Eikmeier and A.J. Rehm (Institute for Microbiology, University of Munster, West Germany) studied the production of citric acid with immobilized *Aspergillus niger*. The spores of the fungus *Aspergillus niger* were entrapped in calcium alginate beads and precultivated with various amounts of nitrogen. During the subsequent citric acid production in shaking cultures, an optimum of acid formation and yield was observed after the precultivation with 100 to 200 mg/L NH_4NO_3. The productivity of the immobilized fungus was found to be 1.5 times higher than in the case of free pellets. Outgrowing of free mycelia could be provided by increasing the ratio of particle-volume:medium-volume, using a 1-L air-lift fermenter, which increased the productivity twice as much as obtained in shaking culture.

The production of ethanol with immobilized *Zymonas mobilis* cells was described by J. Klein and B. Kressdorf (Institute of Technical Chemistry, Technical University, Braunschweig, West Germany). They studied this bacterium since it shows higher ethanol production and sugar consumption as well as a lower growth rate as compared to yeast. Another advantage over yeast is the anaerobic growth, which is of special interest for its use in the immobilized state. By immobilizing *Zymonas mobilis* the researchers increased the cell density, and continuous operation at high dilution rate without washout could be obtained, leading to higher reactor productivity. The maximum reactor productivity using *Zymonas* was twice the value obtained for yeast. The immobilized cell system has the advantage of reliable cell retention, and costly and complicated cell recycle systems can be eliminated by using the entrapment method. A preparation which provides extremely mild conditions is the entrapment in polyelectrolyte gels like calcium algenate and carragenan. Klein and Kressdorf also designed a three-stage cascade with

two fluidized bed reactors and one packed bed reactor. The purpose was to overcome the problems of considerable gas holdup, which is typical for packed bed reactors, and to optimize the reactor configuration in consideration of the kinetics of ethanol formation. They demonstrated an increase of productivity in comparison to packed bed systems.

Applied Genetics

Recombinant DNA technology enables the synthesis of valuable proteins by microorganisms. Plasmids, in particular the relatively small, multicopy plasmids, are widely used for cloning bacterial and eukaryotic genes and amplifying these genes as well as their products. A prerequisite for the use of plasmids in recombinant DNA technology is the stable maintenance of these vectors in bacterial cells. Recently, biochemical and molecular genetic studies have resulted in more insight into the genes and sequences involved into the different processes that are responsible for plasmid maintenance, such as plasmid replication and its controls, and incompatibility between replicons. Studies of the systems that control plasmid copy number have enabled the construction of suitable plasmid cloning vectors--for example, plasmids with a temperature regulated copy number. Furthermore, sequences have been identified in the plasmid genome that are essential for the conjugative transfer of plasmids from one bacterial cell to another. Characterization of these transfer regions and subsequent cloning of these determinants have resulted in the availability of cloning systems in which the mobilization of the plasmid can be switched on and off. Thus, a wide variety of cloning systems are now available for genetic engineering in prokaryotes, while in eukaryotes, corresponding developments have been restricted to a small number of organisms. One of the main difficulties has been that, in contrast to bacteria, plasmids which may be useful for the construction of vectors have been described only recently. Since many of the applications of recombinant DNA technology are concerned with eukaryotic genes and their expression--i.e., production of the gene-coded eukaryotic protein in sufficient amounts to be commercially feasible--genetic engineering in eukaryotes may prove to be easier and more efficient than in prokaryotes. The reasons are as follows: (1) the prokaryotic systems require extensive manipulation to produce a foreign--i.e., eukaryotic--protein, and (2) post-translational modification--i.e., glycosylation--does not occur in genetically engineered prokaryotes, whereas it does take place in eukaryotes.

There were a large number of oral and poster sessions at the congress which dealt with the use of recombinant DNA technology for products with potential industrial application as well as various approaches for enhancing the efficiency of genetic engineering systems. However, it is only possible to present a summary of selected topics in this report.

The orderly process of transforming genes cloned at the bench scale into industrial processes depends on maintaining the genetic stability of the recombinant organisms; many researchers have found that the increased productivity of the gene product is closely related to increased genetic stability. D. Dewey, Y. Ryu, and R.S. Siegel (University of California, Davis, California) dealt with this problem by the application of a two-stage continuous culture to increase the stability and productivity of recombinant organisms containing temperature-derepressible plasmids using the PL plasmids as a model system. Their approach to dealing with the instability of the cloned gene was to separate the growth from the product formation which can be considered a consequence of gene expression. Also, temperature-sensitive mutants offer the possibility of derepression without the need to control the level of inducers or derepressors. The two-stage continuous culture maintained a high specific productivity from the derepressed plasmid and enhanced the stability considerably as compared to

a single-stage continuous culture system.

F. Srienc, J. Campbell, and J. Bailey (California Institute of Technology, Pasadena, California) used a powerful new experimental method based upon flow cytometry to investigate two different recombinant yeast strains. One strain contains a plasmid in which replication function is provided by a cloned sequence from the yeast 2-μm plasmids. A cloned chromosomal autonomous replication sequence provides autonomous plasmid replication in the second strain. The purpose of this study was to obtain information about plasmid replication and partitioning in the yeast *S. cerevisiae* because, in contrast to *E. coli* plasmids, relatively little is known about yeast plasmids and the sequences which regulate replication and segregation. Furthermore, this organism is a model eukaryote and has industrial potential as a host for genetic engineering applications. The productivity of a recombinant microbial population for the desired cloned-gene product depends upon several factors, with the population content of cloned genes being a major determinant. The flow cytometry method provides extremely rapid characterization of a cell population in terms of the distribution of the single cell parameter which is measured. Therefore, one can select readily the most stable plasmid for production of the desired closed-gene product.

C.P. Hollenberg and M. Wilhelm (University of Düsseldorf, West Germany) described a first step toward the construction of a xylose-fermenting yeast strain, i.e., the cloning of the *B. subtilis* xylose isomerase gene in *E. coli* and its location in the xylose operon. The purpose of this study was to develop a method for producing a xylose-fermenting yeast. The yeast strain *S. cerevisiae* is unable to ferment xylose to ethanol, so these investigations are using genetic engineering techniques to eventually produce xylose-fermenting *S. cerevisiae*. Since D-xylose is among the most abundant carbohydrates derived from plant biomass and wood, an abundant supply of xylose is available for its conversion to ethanol, an important industrial compound. Thus, xylose-fermenting yeast constitutes an approach toward improvement in the efficiency and cost of the production of ethanol by fermentation.

Another approach to the improved production of ethanol reported by P. Margalith and R. Legman (Technion, Federal Institute of Technology, Israel) is the formation of a new hybrid of yeast which combines the characteristics of an osmotolerant organism with that of a highly fermentative yeast. In this work, protoplasts of auxotrophic mutants of *S. cerevisiae* and the osmotolerant *S. mellis* were fused. Biochemical and genetic analyses of the hybirds produced were also carried out. Of the stable hybrids obtained, about 35 percent exceeded the fermentative capacity of the parent strains at high sugar concentrations. One strain (No. 37) produced about 13.6 percent (W/W) ethanols after 72 hours of fermentation in comparison with 9 percent of the control *S. cerevisiae* strain. The fusion between fermentative and osmotolerant yeasts could become a valuable technique for the improvement of industrial ethanol-fermenting organisms.

P.M.A. Nybergh (Research Laboratories of the Finnish State Alcohol Company, Alko Ltd., Helsinki, Finland) reported on the production of α-amylase by *B. subtilis* containing a cloned α-amylase gene. This bacterial enzyme is currently used to liquify starch, which is the first step in the hydrolysis of starch in the grain alcohol and starch sugar industries. The purpose of this study was to optimize production media and conditions for industrial production of α-amylase and to find out possible problems when recombinant DNA strains are used as industrial producers. The α-amylase gene was transferred from a traditionally developed *B. amyloliquefaciens* VTT-E7308, which is a good industrial source of α-amylase, to *B. subtilis* using the plasmid PUB110. The new *B. subtilis* strain IHO 6270 produced about five times more α-amylase than the

donor strain and about 2500-fold more than the wild type *B. subtilis* Marburg strain. Some problems remain in scaling up the recombinant DNA strain (*B. subtilis* IHO 6270) to the industrial scale. However, it appears highly likely that cheap bulk enzymes can be produced by recombinant DNA strains.

T. Shibata, H. Watake, K. Nakagawa, T. Iino, T. Kaneko, and T. Ando (Riken Institute, Saitama, Japan) described a site-specific endonuclease (*Endo. Sce I*) produced by the yeast eukaryote *S. Cerevisiae*, which is different from prokaryotic endonucleases. These investigators have already found five other eukaryotic endonucleases. Their data suggest that the activity of *Endo. Sce I* is controlled so the enzyme can play a role in some cellular functions at the specified growth phase, such as genetic recombination at meiosis. Although more than 200 species of site-specific endonucleases, including restriction endonucleases, have been found in various species of prokaryotes, these researchers have been among the first to find eukaryotic site-specific endonucleases.

Plasma-encoded degradation of aromatic hydrocarbons in *Pseudomonas* was described by E. Galli, G. Bestetti, P. Barbieri, and G. Baggi (Department of Biology, Section of Genetics and Microbiology and the Institute of Agrarian Microbiology, University of Milan, Italy). The bacteria belonging to genus *Pseudomonas* are able to metabolize a large number of natural and synthetic organic compounds. The purpose of this study was to obtain information on the genetic organization and regulation of catabolic pathways in *Pseudomonas* in order to be able to manipulate key metabolic functions for use of these organisms in biotechnological applications such as pollutant degradation, energy production, and synthesis of new compounds of industrial interest. The researchers presented detailed studies about the functions of a catabolic plasmid which encodes enzymes for the use of 1, 2, 4-trimethylbenzene in a strain of *Pseudomonas putida* TKM3, isolated from enrichment cultures in the presence of this compound as the only carbon source.

Several reports were presented in which recombinant DNA methods were used for gene amplification and/or production of compounds for eventual use in human as well as animal diseases. H. Hauser, H. Dinter, and J. Collins (Institute for Biotechnology Research, Braunschneig, West Germany) described the construction of interferon-producing mouse cell lines by genetic engineering. The studies concerned the transformation of several mammalian cell lines with the original and a modified beta-interferon-genomic DNA. This resulted in the selection of a mouse cell line which was used in a scaled-up fermentation process to obtain glycosylated human beta interferon.

Another example of the industrial application of recombinant DNA technology for human use was presented by B. Holmström (Biochemical Department, Research and Development, Kabivitrum AB, Stockholm, Sweden). Genentech Inc. in the US was contracted by Kabivitrum to construct bacteria that could synthesize human somatotropin (growth hormones used for the treatment of pituitary dwarfism). Holmström and his group used the genetically engineered bacteria from Genentec Inc. for large-scale production of human somatotropin. A fermentation plant with a 3000:1 ferementer was built in Strängras, 80 km from Stockholm. The purified product, methionyl somatotropin, is now being tested in the clinic following preclinical tests.

A report on the cloning and expression of a synthetic gene for the human insulin-like growth factor-1 (IGF-1) was presented by A. Eimblad, L. Fryklund, L.-O. Hedén, E. Holmgren, S. Josephson, M. Lake, B. Löwenadler, G. Palm, and A. Skottner-Lundin (Department of Chemistry and Molecular Biology, Kabigen AB, Stockholm, Sweden, and the Peptide Hormone Laboratory, Kabivitrum AB, Stockholm). IGF-1 is a peptide hormone homologous to the structure of insulin and showing somatomedin activity *in vitro*. Recent *in vivo* studies have shown growth stimulation of hypophysectomized rats in a dose-dependent manner. By cloning the

gene for IGF-1 and production of the protein, a source is available in significant amounts for antibody production and for studies of its biological significance.

E. Breyel, G. Morella, B. Aufmkolk, R. Frank, H. Blöcker, and H. Mayer (Institute for Biotechnology Research, Genetic Division and DNA Synthesis Group, Braunschweig, and the Medical University of Hannover, West Germany) described the construction of bacterial plasmids which direct the synthesis of biologically active, mature, human parathyroid hormone (PTH) in *E. coli*. This hormone, a protein, is the principal homeostatic regulator of blood calcium and at low concentration stimulates bone formation. Human PTH is effective in the treatment of osteoporosis and is being tested in the treatment of bone fractures. Therefore, the production of PTH by genetic engineering will provide a much cheaper source of this hormone than is presently available by isolation from parathyroid glands.

M. Brockhuijsen, T. Blom, C.A.M.J.J. van den Hondel, M. Kottenhagen, A. Kuijvenhoven, P.H. Pouwels, and B.E. Enger-Valk (Medical Biological Laboratory, TNO, Rijswijk, The Netherlands), and S.J. Bartelings and R.A. Meloen (Central Veterinary Institute, Lelystad, The Netherlands) reported on the construction of expression plasmids containing antigenic determinants of foot and mouth disease virus (FMDV). This virus causes a disease of domestic livestock and prevention of FMDV is of considerable economical importance. Vaccines are produced, at present, by inactivation of virus grown in baby hamster kidney cells or bovine tongue epithelium. The problems associated with the production as well as the increasing demand for vaccine, especially in underdeveloped countries, make the recombinant DNA technique attractive for producing large quantities of VP, the viral polypeptide capsid which contains the main antigenic determinants of the viron. Also, it provides the means of producing (parts of) VP, proteins with different, possibly more antigenic determinants for the development of vaccines.

K.P. Koller, J. Engels, and E. Uhlmann (Hoechstag, Frankfurt, West Germany) presented data on gene amplification and over-production of the α-amylase inhibitor (Hoe 467, tendamistat) in *Streptomyces tendae*. The purified protein has been shown to inhibit intestinal starch degradation, thus strongly influencing blood sugar levels in humans, and is therefore considered to be of therapeutic value in treating metabolic diseases like diabetes mellitus and obesity. Previous large-scale purification of the inhibitor for pharmacological examinations was primarily hampered by low yields and formation of melanin by the wild type strain. These investigations have isolated mutant strains of *S. tendae* which were virtually melanin-negative and produced large amounts of inhibitor. At the congress, the researchers presented data on the molecular genetics of one of the mutants, I-9362, elucidating for the first time the correlation between an amplified genomic element and over-production of a protein in a *Streptomyces* strain.

Food and Feed Bioprocesses

M. Hersel, R. Bronnenmeier, and A. Hoffman (Linde Co., Hoellriegelskreuth, West Germany) reported on a single cell protein (SCP) process with high productivity. SCP is important as an additive to improve the nutritive quality of animal feed and is also of economic importance. Linde Co. has a long tradition in biotechnology, and production of SCP is yet another facet of their expanding biotechnology programs.

The process for SCP production by Linde Co. involves certain technical innovations which have resulted in good economy, plant availability, operability, and product purity. The main features are: (1) readily water-soluble carbon sources--for example, ethanol or methanol; (2) use of very productive yeast strains instead of, for example, bacteria--because of the use of yeast,

sterile operation of the fermenters is not required; (3) use of highly oxygen-enriched air, preferably >95 percent oxygen instead of air for gassing of the fermenters; (4) modular assembly; and (5) a thickening step in the first stage which is simple and safe to operate. These features make the process efficient, according to Hersel et al.

To continuously improve the process of SCP production, Linde has built a demonstration plant at its research center in Hoellriegelskreuth. The work has been partly supported by the German Ministry for Research and Technology. For the SCP process, the yeast produced on the basis of ethanol has been tested in animal feeding trials and was proven to be a powerful additive of high biological value when supplemented with methionine. This methionine supplemented yeast protein was found to be used physiologically about 50 percent better than protein in corn. By addition of yeast to corn or corn products (for example, bread), the biological value of the mixtures is much higher than that of corn alone. To achieve this, in most cases, only a small percentage of yeast has to be added so that there is no change in palatability, texture, and taste. This means that addition of yeast can improve the nutritive quality of both food for humans and feed for animals.

C. Kehagis and B.J. Macris (Institute of Technology of Agricultural Products, Lycovrisi, Greece, and the Department of Biology, Nuclear Research Center "Democritos," Attiki, Greece) reported on microbial protein production from *Fusarium moniliforme* cultivated on whey in a pilot-plant fermenter. The large volume of whey produced in many countries constitutes a challenge to the dairy industry to use this environment polluting by-product. Whey is a good source not only of lactose (milk sugar), but also of several vitamins and minerals which can support microbial growth. With the general increasing production of cheese and whey, plus higher animal feed prices and higher cost of environmental pollution, Kehigas and Macris have attacked the problem of finding a solution to surplus whey. In the study described at the congress, the kinetics of growth of *F. moniliforme* in whey in a pilot plant were investigated. The results of this work showed that the growth characteristics and the crude protein content of *F. moniliforme* cultured on a pilot-plant scale on whey lactose are competitive with those reported for other fungi. These results, together with the good appearance and nutritional quality of *F. moniliforme*, make this fungus a potential candidate for the use of the whey by-product of the dairy industry.

Fine Chemicals/Pharmaceuticals--Bioprocesses

R. Lundell, R. Oy, A. Mustranta, E. Kaila, and J. Karppinen (VTT Biotechnical Laboratory, Espoo, Finland) and G.A. Serlachius (Mäntla, Finland) described a novel process for the production of enzymes. The *P. varioti* strain, TPR-220 (a myceliumforming microfungus) used for commercial production of single cell protein at the G.A. Serlachius Corporation's Mäntla pulp mill in Finland was investigated for its ability to produce extracellular enzymes on various media in aerobic batch fermentation. The microfungus was found to produce significant amounts of several enzymes, including α-glucosidose, B-glucosidase, B-xylosidase, and xylanese on a medium containing wheat bran, starch, and nutrient salts. A laboratory fermenter constructed for continuous asceptic fermentation was used for the production of the cell mass of *P. varioti*. The enzyme production fermentation was carried out by means of a second laboratory fermenter which contained a filter for the filtration and washing of the microfungus cell mass. The *P. varioti* cells produced enzymes immediately after the transfer to the second fermenter, and new nutrient solution with production of the enzymes continuing for about 2 days after the transfer. The results of these studies showed that an enzyme production unit could be connected to the single-cell protein factory by using the cells of the microfungus from the continuous

cultivation for the production of enzymes, after which the cell mass could be returned to the single-cell protein unit. The enzyme test-production in the plant at the Mäntta-Pekilo facility was performed in a 400-L fermenter. This procedure combines the production of single-cell protein and extracellular enzymes for industrial and pharmaceutical use in a continuous process which is fast and economical.

Fine Chemicals/Pharmaceuticals--Downstream Processing

A.L. Smeds, A. Veide, and S.O. Enfors (Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden) discussed the large-scale isolation of membrane-bound enzymes (chromatophores) from *Rhodispirillum rubrum* in aqueous two-phase systems. A recent approach in enzyme technology is the use of stabilized subcellular structures. One such example is chromatophores which are membrane vesicles from photosynthetic bacteria with the capacity of cyclic phosphorylation and used for ATP regeneration--for example, in an enzyme reactor. Although chromatophores have been isolated on a laboratory scale, methods for large-scale isolation of such biological particles had to be developed. These investigators were able to accomplish the large-scale isolation of chromatophores by extraction in an aqueous two-phase system of polyethylene glycol and dextran and separation in a tubular bowl centrifuge. The method fulfilled the requirements of: (1) sufficient purity for use in ATP regeneration in enzyme reactions; (2) good yield; (3) stabilizing conditions throughout the isolation procedures; (4) rapid handling; and (5) low costs.

Z. Er-el, D. Klein, E. Battat, and E. Zomer (Fermentation Unit, Hebrew University, Jerusalem, Israel) have optimized and developed lytic enzymes from various microbial sources for the production of intracellular enzymes from yeast and fungi. While extracellular enzymes have been used in the food industry for a long time for their hydrolytic activity, only recently have intracellular enzymes been recognized for their catalytic activity as fungi and yeast possess many unique enzymatic systems. The various physical methods available, or the use of commercially available lytic enzymes such as glusulase and zymolase, were not very effective in disrupting the intracellular enzymes from yeast and fungi. The lytic enzymes developed by these investigators, on the other hand, are active at a wide temperature range (5°C to 50°C) which allows the choice of temperature according to the sensitivity of the intracellular enzyme required. The disruption could be carried out at high cell concentration (over 10 percent by weight) with no decrease in yield. High specific activity and low contamination with nucleic acid allowed the enzyme to be purified by ultrafiltration and column chromatography for final purification. Some enzymes have been produced successfully thus far on a pilot scale from *Aspergullus sp.*, *Candida sp.*, and *Pachysolen tannophilus*.

The possibility of using microorganisms as producers of biologically active substances has increased with the development of recombinant DNA technology. In most cases the product of interest accumulates within the cells which have to be disrupted in order to release the product. A major difficulty in the purification procedure is the clarification of the cell homogenate. Centrifugation and filtration are time consuming and costly. An alternative method is extraction of the product from cell debris in aqueous two-phase systems. For products that are difficult to extract due to low partition coefficient, soluble ligands can be used. A.K. Frej, J.G. Gustafsson, and P. Hedman (Pharmacia Fine Chemicals, Uppsala, Sweden) presented data on the use of chromatography gels which they termed "carrier absorbents" as absorbents in aqueous two-phase systems to improve the efficiency of extraction. Their work demonstrated the use of carrier adsorbents for purification of B-galactosidase directly from an unclarified *E. coli*

homogenate, and separation of the adsorbent-product complex from the cell debris by partitioning in an aqueous two-phase system. Phenyl Sepharose CL-4B manufactured by Pharmacia was used as the carrier adsorbent. These investigators reported that addition of extractable "carrier adsorbent" to cell homogenates was a practical method to circumvent the difficult homogenate clarification step. It was found that removal of the polymers and salt (which were added to induce the phase separation from the product) was easy to carry out. Also, the recovery and degree of purification was determined by the carrier adsorbent rather than the phase system.

3 CONCLUSION

Although there were a large number of oral and poster presentations at the congress, many reports, although interesting, consisted of preliminary data which, in many instances, were inconclusive. Therefore, this report discussed selected presentations which were definitive in the conclusions drawn by the investigators and presented novel approaches to the solution of a wide range of biotechnology research. Since the majority of the talks were by European scientists, it is evident that the European countries are making important contributions to biotechnology in both basic and applied research. Recently, extensive commitments have been made by the governments of most European countries for increased financial aid not only to university research but also to programs fostering cooperation between industry and the academic sector. Therefore, one can expect an increasing emphasis on basic and applied research in biotechnology by European scientists.

APPENDIX:
TOPICS COVERED AT THE CONGRESS

I. PLENARY LECTURES
   1. Fine Chemicals
   2. Present State of Biotechnological Productions of Pharmaceuticals
   3. Biotechnology and Base Chemicals
   4. A Time Table for Commercializing Biomass Refining
   5. New Food Sources: A Challenge for Biotechnology
   6. From Environment to Biotechnology and Vice Versa

II. PANEL DISCUSSIONS
   1. European Community Action in Biotechnology
   2. Technology Transfer to Developing Countries

III. SPECIAL SEMINARS
   1. Mass Cell Culture Technology
   2. Applied Molecular Genetics
   3. Safety in Biotechnology
   4. Immobilized Biocatalysts
   5. Education in Biotechnology
   6. Futuristic Aspects in Biotechnology

IV. WORKSHOPS
   1. Contamination in Fermentation
   2. Physical Properties of Fermentation Liquids
   3. Economics of Biotechnology
   4. Microorganisms Assist in Solution of Environmental Problems
   5. Coenzymes Regeneration and Electro-enzymology

V. GENERAL PAPERS (oral and poster sessions)
   1. Application Topics
      a. Fine Chemicals and Pharmaceuticals
      b. Basic Organic Chemicals and Raw Materials Including Substrates
      c. Fuels and Energy
      d. Food and Feed
      e. Environmental Biotechnology

   2. Fundamental Topics
      a. Applied Microbial Physiology
      b. Applied Genetics
      c. Cell Culture (animals and plants)
      d. Biocatalysts
      e. Bioprocesses
      f. Bioreactors
      g. Downstream Processing
      h. Measurement and Process Control

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